ANALYSIS OF GENETIC MOSAICS OF THE NEMATODE *CAENORHABDITIS ELEGANS*

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ABSTRACT

A new method for producing genetic mosaics, which involves the spontaneous somatic loss of free chromosome fragments, is demonstrated. Four genes that affect the behavior of *C. elegans* were studied in mosaic animals. The analysis was greatly aided by the fact that the complete cell lineage of wild-type animals is known. Two of the mutant genes affect certain sensory responses and prevent uptake of fluorescein isothiocyanate (FITC) by certain sensory neurons. Mosaic analysis indicated that one of these mutant genes is cell autonomous with respect to its effect on FITC uptake and the other is cell nonautonomous. In the latter case, the genotype of a non-neuronal supporting cell that surrounds the processes of the neurons that normally take up FITC probably is critical. The other two mutant genes affect animal movement. Mosaic analysis indicated that the expression of one of these genes is specific to certain neurons (motor neurons of the ventral and dorsal nerve cords are prime candidates) and the expression of the other gene is specific to muscle cells.

THE analysis of genetic mosaics and chimeras has been a powerful tool in developmental genetics, primarily in work with Drosophila and the mouse (for reviews, see GEHRING 1978). In Drosophila, in which the analysis of genetic mosaics has been most extensive, mosaics have been used to elucidate cell lineage and to ascertain the anatomical foci of mutations affecting behavior. In cases in which cellular abnormalities in mutants have been discernible, genetic mosaics have been used to assess the cell autonomy of mutant phenes; cell autonomy implies that the action of a mutation on cell differentiation is intrinsic to the cell, whereas nonautonomy indicates that cell-cell interactions are involved. Such interactions can also be studied by mosaic analysis (see, for example, MEYEROWITZ and KANKEL 1978). Genetic mosaics have also been used to set limits on the times of action of wild-type genes: if a wild-type gene is removed from a cell and as a consequence a descendant cell shows a recessive mutant phenotype, the implication is that the wild-type gene was needed after the time at which it was removed.

The development of the nematode *Caenorhabditis elegans* is under intensive investigation: the complete cell lineage of the wild-type animal has been worked out (SULSTON 1976; SULSTON and HORVITZ 1977; DEPPE *et al.* 1978; KIMBLE and HIRSH 1979; SULSTON *et al.* 1983), and the neuroanatomy of the animal has

been reconstructed from serial section electron micrographs (WARD *et al.* 1975; WARE *et al.* 1975; WHITE *et al.* 1976; ALBERTSON and THOMSON 1976; J. WHITE, personal communication). One reason for the choice of *C. elegans* as a developmental model is its suitability for many methods of genetic analysis (BRENNER 1974; HERMAN and HORVITZ 1980). It would clearly be desirable to add mosaic analysis to the methods available. Genetic mosaics are not needed to work out *C. elegans* cell lineages because they can be followed by direct observation, but the other applications of mosaic analysis, dealing with the cell specificity of gene expression, would be very useful. SIDDIQUI and BABU (1980) have reported the production of *C. elegans* mosaics by X irradiation of embryos heterozygous for *flu-3*, a gene that alters the autofluorescence of intestinal cells under ultraviolet light. The disadvantages of their method are that the frequency of mosaicism is low (less than 0.1% mosaic animals), the radiation (2000 rads) is likely to cause cell death and other abnormalities and the mechanism by which the mosaics arise is not clear. Here, I report a new method for producing mosaics: the spontaneous loss of a free chromosome fragment present as a duplication. Duplication loss thus generates a cell with a normal chromosome complement; because the cell lineages are rigidly specified and completely known, it should be possible with appropriate cell markers to pinpoint precisely the division at which a duplication loss took place and, hence, predict the exact cell composition of the duplication-free clone in the mosaic animal. Free chromosome duplications representing several different regions of the genome have been identified (HERMAN, ALBERTSON and BRENNER, 1976; HERMAN, MADL and KARI 1979; HODGKIN 1980; P. ANDERSON, personal communication; A. ROSE and D. BAILLIE, personal communication). Because of the holokinetic nature of the *C. elegans* chromosomes (ALBERTSON and THOMSON 1982), suitable free duplications of most regions of the genome may eventually become available and help make the approach introduced here more generally applicable.

MATERIALS AND METHODS

Genes, alleles and general procedures: *C. elegans* var. Bristol strain N2 was the wild-type parent for all strains used in this work. The following genes and mutations were used: LG II: *dpy-10(e128);* LG III: *unc-93(e1500);* LG V: *dpy-11(e224);* LG X: *unc-9(e101), let-4(mn105), unc-3(e151), daf-6(e1377), let-1(mn119), unc-7(e139), sup-10(n183,mn219), osm-1(p808).* The derivation of *sup-10(mn219)* is described under *Strain constructions*. The sources of the other mutations are either cited in RESULTS (or under *Strain constructions* in MATERIALS AND METHODS) or they were described by BRENNER (1974). Media, culture and mating techniques were as described by BRENNER (1974) and HERMAN (1978). Genetic nomenclature follows the guidelines described by HORVITZ *et al.* (1979).

Duplications: The derivations and characterizations of *mnDp1(X;V)*, *mnDp2(X;f)* and *mnDp3(X;f)* have been described (HERMAN, ALBERTSON and BRENNER 1976; HERMAN, MADL and KARI 1979). In addition, CHALFIE and SULSTON (1981) have shown that *mnDp2* does not suppress *mec-5* or *mec-4* mutations, which map to the right of the region covered by *mnDp2*, and I have shown that *mnDp2* does not suppress *osm-1(p808)*, which maps near *mec-4*. The free *X* chromosome duplications *mnDp12(X;f)* and *mnDp14(X;f)* were induced, identified and characterized by C. KARI (personal communication) by methods previously described (HERMAN, ALBERTSON and BRENNER 1976) except that γ rays were used rather than X rays. γ Radiation was supplied by ^{137}Cs in a Shepherd irradiator (model 143-45). Doses of 7200 roentgens (r) were used at a dose rate of 600 r/min. Both *mnDp12* and *mnDp14* carry the wild-type alleles of *unc-3*, *daf-6*, *unc-7*, *sup-10* and *osm-1* but not *unc-9*, as judged by their ability to suppress mutations in these genes. *mnDp13(X;f)* is a variant of *mnDp3* that lacks *sup-10+* but carries *osm-1+* (see RESULTS for derivation). The average percentage nullo-

duplication self-progeny of duplication-bearing hermaphrodites determined for each duplication (at least 1000 total progeny per measurement) were 59% for *mnDp2*, 45% for *mnDp3*, 76% for *mnDp12*, 44% for *mnDp13* and 52% for *mnDp14*.

FITC staining: The fluorescein isothiocyanate (FITC)-staining protocol of E. HEDGECOCK (personal communication) was followed: animals were put on an agar growth plate seeded with bacteria to which FITC had been added to a final concentration of 0.1 mg/ml. Worms were removed from the plate 3–12 hr later, put on a seeded plate without dye for at least 10 min and then put on a 5% agar pad on a microscope slide (SULSTON, ALBERTSON and THOMSON 1980) and viewed by epifluorescence.

Genetic mapping of daf-6 *and* osm-1: Fifteen independent Unc-7 non-Unc-3 recombinant self-progeny of *unc-3 unc-7/daf-6* hermaphrodites were picked. Segregants homozygous for the recombinant chromosome (giving no Unc-3 Unc-7 self-progeny) were found in each case. FITC staining was used to ascertain the status of the *daf-6* locus: five chromosomes were *daf-6+* and ten were *daf-6*; thus, *daf-6* maps between *unc-3* and *unc-7*. The *osm-1* gene, previously mapped by CULOTTI and RUSSELL (1978), was further localized by complementation testing against deficiencies *mnDf41*, *mnDf42* and *mnDf43* (for descriptions of these deficiencies and the complementation methods, see MENEELY and HERMAN 1981); *osm-1* complemented *mnDf43* and failed to complement *mnDf41* and *mnDf42*. This places it to the right of *sup-10* (MENEELY and HERMAN 1981).

Strain constructions: Most of the strains were constructed by standard methods that need not be described. The *unc-3 daf-6* double mutant was constructed as follows: Males of genotype *mnDp1-(X;V)/+*; *unc-3 let-1/0* (MENEELY and HERMAN 1981) were mated with *dpy-10; daf-6* hermaphrodites. Wild-type hermaphrodite progeny were picked. Their progeny were screened for viable Unc recombinants, which were picked. Their progeny were then screened by FITC staining for *unc-3 daf-6* homozygotes.

The *let-4 unc-3 osm-1; mnDp13* strain was made as follows. Males of genotype *mnDp1/+*; *let-4 unc-3/0* (MENEELY and HERMAN 1981) were crossed with *dpy-11; unc-3; mnDp13* hermaphrodites. Wild-type hermaphrodite progeny were picked, and those that did not carry *mnDp1* were identified by the absence of pseudolinkage between *dpy-11* and *unc-3*. Wild-type hermaphrodite progeny were picked, and hermaphrodites of genotype *let-4 unc-3; mnDp13* were identified by the absence of viable Unc animals among their self-progeny. Males of genotype *let-4 unc-3/0; mnDp13* were then mated with *dpy-11; unc-3 osm-1* hermaphrodites, and wild-type hermaphrodite progeny were picked. More than 100 wild-type hermaphrodite self-progeny of these animals were then picked. Self-progeny broods produced by these animals in which both viable Unc and arrested larvae were represented were identified and stained with FITC. A brood in which all Unc animals did not stain with FITC (but wild-type animals did stain) was found, and from it a wild-type descendant of genotype *let-4 unc-3 osm-1; mnDp13* was readily identified.

The *unc-93; unc-3 sup-10 osm-1* mutant was generated as follows. Rather than try to produce the *sup-10 osm-1* double mutant by recombination, a new spontaneous *sup-10* mutation was sought in an *unc-93; osm-1* background, which was produced by standard methods. The *unc-93; osm-1* strain was grown on dozens of 100-mm agar plates to select for spontaneous reversion of the Unc-93 phenotype. Revertants were then tested for the possession of an extragenic suppressor mutation by crossing them with N2 males, picking wild-type hermaphrodites from plates in which mating had been efficient (as judged by the presence of many male progeny) and looking for the segregation of Unc-93 self-progeny. In those cases in which an external suppressor was present, linkage of the suppressor to *osm-1* was tested by FITC staining of both Unc-93 and non-Unc-93 segregants. Among the first five revertants identified, two carried an external suppressor, and one of these (*mn219*) was closely linked to *osm-1*. Finally, a complementation test established the allelism of *mn219* and *sup-10(n183)*. Next, *unc-93/+; sup-10 osm-1/unc-3 osm-1* hermaphrodites were produced by crossing *mnDp1/+; unc-3 osm-1/0* males with *unc-93; sup-10 osm-1* hermaphrodites. Unc-93 segregants of these were picked, and then wild-type self-progeny (homozygous for both *unc-93* and *sup-10*) of the Unc-93 animals were picked. Finally, an Unc-3 segregant (genotype *unc-93; unc-3 sup-10 osm-1*) was identified.

RESULTS

FITC staining of amphid and phasmid neurons: When a living wild-type *C. elegans* animal is exposed to a solution of FITC, six neurons in each of a pair of sensilla

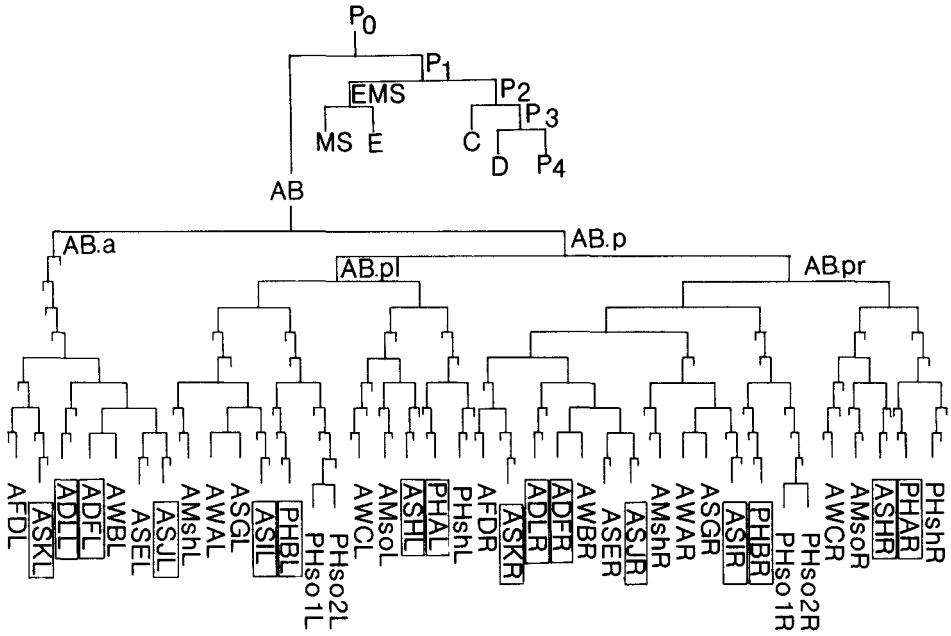

FIGURE 1.—Lineages of cells comprising amphids and phasmids (SULSTON *et al.* 1983). Lineage tree conventions and cell nomenclature follow SULSTON and HORVITZ (1977), DEPPE *et al.* (1978) and SULSTON *et al.* (1983). The fertilized egg is called P_0. Each of four successive asymmetric cleavages generates a larger somatic precursor cell (AB in the first cleavage) and a smaller P cell (DEPPE *et al.* 1978). P_4 is the precursor of the germ line. AB.a and AB.p are the anterior and posterior daughters of AB, respectively; and AB.pl and AB.pr are the left and right daughters of AB.p, respectively. Nearly all unmarked divisions shown give anterior and posterior daughters; an anterior duaghter is represented by a left branch in the lineage tree. Thus, for example, the right-most cell shown in the diagram (PHshR) has the lineage designation AB.prpppapaa. Lineages leading to cells not comprising the amphids and phasmids have been terminated in this figure; see SULSTON *et al.* (1983) for complete lineages. Differentiated cell names beginning with A and PH refer to amphid and phasmid, respectively; sheath cell is abbreviated "sh" and socket cell is abbreviated "so"; all other cells are neurons. The cells that are stained in wild-type animals by FITC (E. HEDGECOCK, personal communication) are outlined.

in the head, called amphids, and two neurons in each of a pair of sensilla in the tail, called phasmids, take up the dye and can be viewed, in the live animal, by fluorescence microscopy (E. HEDGECOCK, personal communication). Neuron cell bodies as well as processes fill with dye, and it is the cell bodies that can be most readily distinguished. Each amphid consists of 12 neurons and two non-neuronal cells, a sheath cell and a socket cell (WARD *et al.* 1975; WARE *et al.* 1975). Each phasmid consists of two neurons, a sheath cell and two socket cells (SULSTON, ALBERTSON and THOMSON 1980). The lineages of all of these cells are indicated in Figure 1, which also shows which cells are stained by FITC. Each sensillum has a channel open to the external environment. The channels are formed by the sheath and socket cells, which surround neuronal processes located in the channels. Certain mutants that are abnormal with respect to chemotaxis, male mating efficiency, ability to avoid high osmotic pressure or ability to form dauer

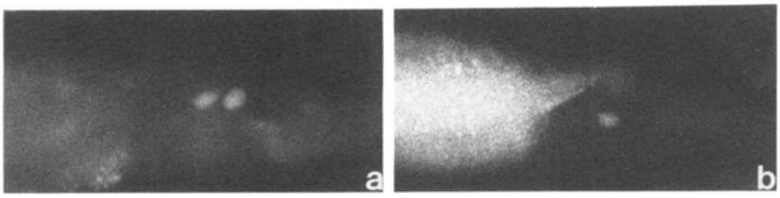

FIGURE 2.—Fluorescence micrographs showing lateral views of cell bodies of (a) two neurons of one phasmid of a wild-type animal stained with FITC and (b) one stained phasmid neuron in an *osm-1* mosaic animal. The large area of fluorescence is part of the intestine. Animals were anesthetized with 1-phenoxy-2-propanol (SULSTON, ALBERTSON and WHITE 1980) for photography. Magnifications in both photographs are ×560.

larvae (an alternative to the normal third stage larvae) in response to starvation or overcrowding have been shown to be abnormal in the structure of amphids or phasmids (WARD 1976; LEWIS and HODGKIN 1977; ALBERT, BROWN and RIDDLE 1981; L. PERKINS, E. HEDGECOCK, N. THOMSON and J. CULOTTI, personal communication), presumably reflecting abnormal sensory function in these mutants. Furthermore, certain mutations, including alleles of *osm-1* (CULOTTI and RUSSELL 1978) and *daf-6* (ALBERT, BROWN and RIDDLE 1981), abolish the FITC staining of amphid and phasmid neurons (E. HEDGECOCK, personal communication).

Mosaic expression of osm-1 *and* daf-6: Hermaphrodites homozygous for *unc-3* and *osm-1* on the *X* chromosome and carrying a free duplication bearing the wild-type dominant alleles of these two genes segregate two principal classes of self-progeny: wild-type hermaphrodites, which carry the duplication, and uncoordinated (Unc-3) FITC-nonstaining hermaphrodites, which do not. Suppose, however, that there has been somatic loss of the free duplication such that the *unc-3*+ function has been provided in the necessary cells but *osm-1*+ function required for normal sensilla staining is missing in some cells. To look for such animals I have screened non-Unc-3 progeny of *unc-3 osm-1; mnDp12* hermaphrodites for their patterns of FITC staining. As a control, I have used a strain that has in place of the free duplication a duplication of the same region of the *X* chromosome translocated to an autosome (*mnDp1*), which should be mitotically stable. Eight percent of the free duplication-bearing animals screened (and none of the control animals) showed absence of staining of one phasmid neuron; see Figure 2 and Table 1. This is the result one would expect for cell autonomous expression of *osm-1* if each nonstaining cell lacked the free duplication. For example, an animal in which PHBR but not PHAR stained presumably would have undergone duplication loss between AB.prp and AB.prpppaapp; see Figure 1. (No attempt was in fact made in these experiments to determine which of the two phasmid neurons—PHA or PHB—was not staining in particular instances.) There were also animals in which neither neuron of a given phasmid stained (Table 1), but such animals were found at about the same frequency in the control strain; therefore, the events responsible for these animals are not attributed to duplication loss. The lack of staining of both neurons of a phasmid has also been observed in the N2 strain and might be due to occasional clogging

TABLE 1

FITC staining of non-Unc-3 hermaphrodites

Zygote	No. of indicated sensilla not staining						Total no. animals scored
	(1/2)LP	(1/2)RP	LP	RP	LA	RA	
unc-3 osm-1; mnDp12(X; f)	10	6	3	4	0	0	200
mnDp1(X; V)/dpy-11; unc-3 osm-1	0	0	5	5	0	0	208
N2	0	0	2	0	0	0	306
unc-3 daf-6; mnDp2(X; f)	0	0	11	18	8	12	316
mnDp1(X; V)/+; unc-3 daf-6	0	0	3	2	0	0	239

Abbreviations used: L = left, R = right, P = phasmid, A = amphid. The symbol (1/2)LP signifies staining of one neuron of left phasmid. In only three animals did more than one sensillum show lack of staining: all were *unc-3 daf-6; mnDp2* zygotes with two sensilla affected (RP and LA; RP and RA; LA and RA).

of a phasmid channel. The frequency of phasmid nonstaining may be higher in the duplication-bearing strains than in N2 (Table 1); it is possible that this is related to the fact that duplication-bearing animals tend to be slightly smaller and less vigorous than N2. In any case, this is not a problem in the staining of amphid neurons: none of the free duplication-bearing animals or the control animals showed absence of staining of all six neurons of an amphid. No attempt was made to score absence of staining of a single amphid neuron, which is technically more difficult than for the phasmid neurons.

A similar set of experiments with *daf-6* in place of *osm-1* gave quite different results (Table 1). In this case, the free duplication-bearing strain (carrying *mnDp2*) showed no absence of staining by single phasmid neurons, but whole sensilla, both amphids and phasmids, frequently showed lack of staining. When allowance is made for the control, which again gave some animals with a nonstaining phasmid, the incidence of nonstaining of any particular sensillum was about 3%. A single event of duplication loss appears to lead to complete absence of staining by one sensillum; the few cases of lack of staining by two sensilla are consistent with the occurrence of double events. The observed pattern of staining is inconsistent with cell autonomous expression of *daf-6* with respect to FITC staining. For example, for all stainable neurons of the left amphid to lack the duplication through a single loss event, the loss would have to occur in the cell called AB (Figure 1), in which case none of the neurons in any of the sensilla would carry the duplication. Indeed, on the basis of their electron microscopic work with the *daf-6* mutation, ALBERT, BROWN and RIDDLE (1981) concluded that the primary defect is in the sheath cell, which accumulates vesicles and enlarges in such a way that the channel to the outside is completely closed. By contrast, the amphid channels in *osm-1* animals remain open to the outside, but the channel neurons are foreshortened and show other abnormalities (L. PERKINS, E. HEDGECOCK, N. THOMSON and J. CULOTTI, personal communication). Therefore, it seems likely that a sheath cell lacking *daf-6+* would block staining by the whole sensillum. Thus, according to this interpretation, for example, an animal in which only the left amphid did not stain could have undergone duplication loss between AB.pla and AB.plaapaapp (Figure 1). Also consistent

TABLE 2

FITC staining of Unc-3 duplication mosaics

	No. of animals with indicated sensilla not staining		Unc-3 duplication mosaic per duplication-bearing sib[a]
	LP RP LA RA	LP RP RA	
unc-3 osm-1; mnDp3(X; f)	6	1	7/8000
unc-3 osm-1; mnDp13(X; f)	1	0	1/700
let-4 unc-3 osm-1; mnDp13 (X; f)[b]	1	2	3/15,000
unc-3 daf-6; mnDp2(X; f)	5	0	5/900

See footnote to Table 1 and text.

[a] For the first two strains and the last strain listed, Unc-3 animals were picked, usually five or ten per plate. From a plate with young non-Unc progeny, the Unc-3 parents were picked individually to identify the one animal responsible. The observed frequency of Unc-3 duplication mosaics per Unc-3 animal for each strain was multiplied by the appropriate ratio of Unc-3 to wild-type animals characteristic of the duplication (see MATERIALS AND METHODS) to obtain the ratios given in this column.

[b] The recessive larval lethal mutant gene *let-4* was used to select against Unc-3 animals devoid of *mnDp13* (which carries *let-4+ unc-3+ osm-1+*). The fact that fertile animals were found whose cells descending from AB or AB.p lacked *let-4+* indicates that the *let-4+* gene is not absolutely required by the cells; on the other hand, the relatively low incidence of these duplication mosaics may reflect a low level requirement for *let-4+* product among AB descendants, which is occasionally inherited in sufficient amount from P_0.

with the results is the possibility that a socket cell lacking *daf-6+* would abolish staining.

Anatomical focus of unc-3 *action*: The mosaic animals described so far were all non-Unc-3. I now ask if it is possible to identify animals that are Unc-3 by virtue of somatic duplication loss. Unc-3 descendants of free duplication-bearing hermaphrodites otherwise homozygous for either *unc-3 osm-1* or *unc-3 daf-6* were picked and screened for their ability to give rise to non-Unc-3 self-progeny, which would indicate retention of the duplication in the germ line. Such animals were found and exposed to FITC (Table 2). In every case listed, at least one non-Unc-3 offspring was shown to stain normally with FITC, confirming that the duplication still carried *osm-1+* (as well as *unc-3+*). Eight of the 11 Unc-3 animals mosaic for *osm-1+* showed complete absence of FITC staining; the remaining three showed staining of left amphid neurons only. Assuming that *osm-1* is cell autonomous with respect to FITC staining and that the Unc-3 animals have undergone a single loss of the free duplication, the losses must have occurred at AB in the eight animals showing no staining and AB.p in the other three (Figure 1). All five of the Unc-3 animals mosaic for *daf-6+* showed complete absence of staining. Assuming that *daf-6* acts on sheath (or socket) cells, this result is consistent with loss of the free duplication at either AB or AB.p since all sheath (and socket) cells derive from AB.p. None of the losses could have occurred later than AB.p; otherwise, at least two sensilla would have stained

(Figure 1). Thus, in all 16 animals made Unc-3 by virtue of somatic duplication loss, the duplication loss occurred no later than at AB.p. We have seen that later duplication losses occur among non-Unc-3 animals (Table 1). This suggests that more than one descendent of AB.p must lack *unc-3*+ in order to give rise to an Unc-3 animal; that is, *unc-3* has a diffuse focus of action, which seems to be at least primarily localized among the descendents of AB.p.

This interpretation suggests that a loss occurring after AB.p might give a semi-Unc-3 phenotype. Clear examples of semi-Unc-3 animals have been found. The distinction between Unc-3 and semi-Unc-3 animals was obvious. When touched on the head, Unc-3 animals do not back up but coil their tails. By contrast, semi-Unc-3 animals are able to back up, albeit in uncoordinated fashion; this phenotype is very similar to that conferred by a weak allele of *unc-3, e54*. Many of the FITC-staining patterns found for semi-Unc-3 animals were consistent with duplication loss occurring at AB.pl or AB.pr (Table 3). In the case of the *osm-1*-marked animals, loss at AB.pl should lead to absence of staining by both left phasmid neurons, and loss at AB.pr should lead to absence of staining by all right phasmid and right amphid neurons. For the *daf-6*-marked animals, on the other hand, loss at AB.pl should lead to absence of staining by all left phasmid and left amphid neurons, and loss at AB.pr should lead to absence of staining by all right amphid and right phasmid neurons. These predicted patterns were observed. In addition, many of the semi-Unc-3 animals showed later duplication losses or possibly double-event losses. The results thus indicate that the descendants of AB.pl and AB.pr are contributing about equally and additively to the Unc-3 phenotype.

Focus of sup-10 *action*: The mutation *unc-93(e1500)* confers a phenotype very different from Unc-3: the animals have long bodies and assume abnormal postures when not moving; they move in a slow and uncoordinated fashion, and they recoil and then quickly relax when touched on the head (GREENWALD and HORVITZ 1980). Because a mild disorganization of the pattern of birefringence of body wall muscles is apparent, GREENWALD and HORVITZ (1980) have concluded that *unc-93* animals are defective in muscle. The mutants are also egg-laying deficient, presumably because of defective vulval and uterine muscles (TRENT, TSUNG and HORVITZ 1983); as a result, progeny hatch inside the parent and devour it, giving small brood sizes (about 30 animals compared with about 300 for N2). All of the *unc-93* phenes are suppressed by the recessive suppressor *sup-10* (GREENWALD and HORVITZ 1980).

Because the free duplication *mnDp3* carries the dominant allele *sup-10*+ (as well as *unc-3*+), hermaphrodites of genotype *unc-93 III; unc-3 sup-10(n183) X; mnDp3* are Unc-93 and segregate two types of self-progeny: Unc-93 hermaphrodites, which carry the duplication, and Unc-3 non-Unc-93 hermaphrodites, which do not. On the assumption that *sup-10* expression is specific to muscle cells, a mosaic animal in which *mnDp3* is retained among AB descendants (for *unc-3*+ function) but lost from body muscle cells should show wild-type movement. The lineages of body muscle cells given in Figure 3 show that there is only one way a single somatic duplication loss will lead to a large majority of body muscle cells lacking *mnDp3*: loss by P_1, which is an ancestor of all body muscle cells but one.

TABLE 3

FITC staining of semi-Unc-3 hermaphrodites

Zygote	No. of animals with indicated sensilla not staining										
	None	(1/2)LP	(1/2)RP	LP	RP	LP RP	LP LA	RP RA	LP LA RP	LP LA RA	RP RA LP
unc-3 osm-1; mnDp12(X; f)	2	1	1	3	0	0	0	3	0	0	1
unc-3 osm-1; mnDp14(X; f)	0	1	0	1	0	0	0	1	0	0	0
let-4 unc-3 osm-1; mnDp13(X; f)	1	1	0	1	0	0	0	2	0	0	0
unc-3 daf-6; mnDp2(X; f)	3	0	0	6	3	2	12	9	1	3	2

See footnote to Table 1. The incidence of semi-Unc-3 animals was estimated at approximately 0.3% per duplication-bearing sib for the *mnDp12* and *mnDp14* strains, approximately 0.05% for the *mnDp13* strain and approximately 1% for the *mnDp2* strain. More than 1100 progeny of *mnDp1/+; unc-3 daf-6* hermaphrodites were screened for semi-Unc-3 animals and none was found.

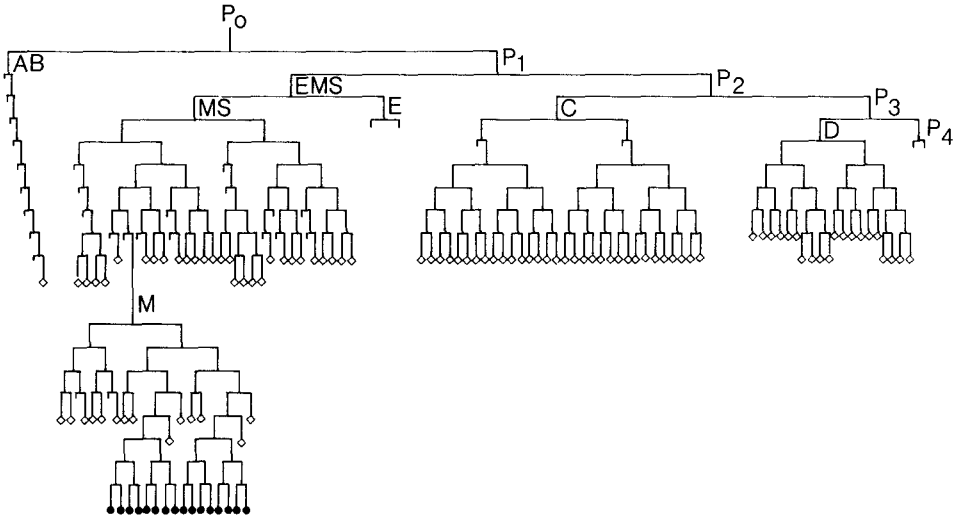

FIGURE 3.—Lineages of the 95 body wall muscle cells (◇) and 16 vulval and uterine muscle cells (●) present in the adult hermaphrodite. The newly hatched animal has 81 body muscle cells: 20 from D, 32 from C, 28 from MS and one from AB (SULSTON *et al.* 1983). The mesoblast called M gives rise postembryonically to 14 additional body muscle cells, as well as to the vulval and uterine muscle cells (SULSTON and HORVITZ 1977). P_4 is the germ line precursor cell.

All vulval and uterine muscles derive from P_1, so that an animal produced in this way should also be wild type with respect to egg laying and, hence, brood size. All gametes also derive from P_1; therefore, all self-progeny of such a wild-type duplication mosaic should be Unc-3 non-Unc-93. Four animals satisfying these predictions (*i.e.*, non-Unc and giving at least 200 self-progeny per animal, all Unc-3) were found among about 8200 *mnDp3*-bearing sibs. In addition, three animals satisfying these predictions were found among about 5500 *mnDp3*-bearing progeny of *unc-93 III; unc-3 sup-10(mn219) osm-1 X; mnDp3* hermaphrodites. The latter three animals were also tested for FITC staining and found to be wild type, as expected since the *osm-1*+ function would have been retained among the AB descendants. The overall incidence of the wild-type *mnDp3* mosaics in these experiments (seven/13,700) agrees with the estimated frequency of *mnDp3* loss by AB (six/8000; Table 2), the sister of P_1.

Another class of wild-type segregant among the progeny of *unc-93; unc-3 sup-10; mnDp3* (and *unc-93; unc-3 sup-10 osm-1; mnDp3*) was also found, at a frequency of about 0.15% among *mnDp3*-bearing animals. These gave wild-type as well as Unc-3 (but not Unc-93) animals among their self-progeny. In every case, it was apparent from the self-progeny ratios that the wild-type animals carried a single *unc-3*+ allele and that it was carried by a free duplication. Thus, it appeared that the wild-type segregants were formed by virtue of loss of *sup-10*+ (and not *unc-3*+) from the free duplication, either by recombination with the *X* chromosome or by mutation of the duplication. One such duplication was shown directly to suppress *unc-3* but not *sup-10*, as predicted: a duplication-bearing (non-Unc) stock was crossed with N2 males, wild-type male progeny were mated with *unc-93; unc-3 sup-10* hermaphrodites, wild-type hermaphrodite progeny were picked

and they were seen not to segregate Unc-93 offspring. Six presumably altered free duplications generated in *unc-93; unc-3 sup-10; mnDp3* animals were tested as follows for their ability to suppress *osm-1*, which is closely linked to *sup-10*. Each duplication-bearing (non-Unc) stock was mated with N2 males; wild-type male progeny were picked and crossed with *dpy-11 V; unc-3 osm-1 X* hermaphrodites, and wild-type male progeny were picked and assayed for their FITC stainability. Two of the six duplications failed to provide *osm-1*+ function. Because the events producing these altered duplications occurred in a strain that carried *osm-1*+ *X* chromosomes, it seems likely that, in at least two cases, the loss of *sup-10*+ occurred through the formation of a deficiency that simultaneously led to loss of the nearby *osm-1*+ gene.

There is another phenotype expected to be produced through somatic duplication loss in *unc-93; unc-3 sup-10; mnDp3* hermaphrodites. If duplication loss occurs after P_1 in a cell that is a precursor to the vulval and uterine muscle cells (Figure 3), the resulting mosaic would be expected to be largely Unc-93 with respect to movement but wild type with respect to egg laying. These animals would retain *mnDp3* in their germ lines and, thus, should give both Unc-93 and Unc-3 self-progeny. Animals satisfying these predictions were also found; their relatively high incidence (15/5000) probably reflects the many divisions in which duplication loss can occur (presumably anywhere between EMS and one of the daughters of M; see Figure 3). I have also picked out three Unc-93 egg layers among the progeny of *unc-93; unc-3 sup-10 osm-1; mnDp3* hermaphrodites and shown that they stained normally with FITC, as expected.

As a control for the experiments involving *sup-10*, I have looked for both wild-type hermaphrodites giving only Unc-3 self-progeny and Unc-93-moving egg-laying-proficient animals, as defined before, among the progeny of *unc-93 III;mnDp1(X;V)/+ V; unc-3 sup-10 X* hermaphrodites. Again, because *mnDp1*, which carries *sup-10*+ and *unc-3*+, is translocated to an autosome and is not free, no mosaic animals were expected. No wild-type hermaphrodite giving only Unc-3 self-progeny was found among 13,000 *mnDp1*-bearing hermaphrodites, and no egg layer was found among 5000 Unc-93 animals scored for egg laying. These control experiments indicate that the exceptional animals identified in the *mnDp3* experiments were not simply the result of occasional incomplete expression of the single *sup-10*+ allele but were in fact genetic mosaics. The experiments with *sup-10* mosaics thus support the conclusion of GREENWALD and HORVITZ (1980) that the action of *sup-10* is specific to muscle cells and provide evidence that the vulval and uterine muscles are able to function in egg laying even when the body wall muscles are largely abnormal; the results also indicate that *unc-3*+ and *osm-1*+ functions are not required in non-AB cells.

DISCUSSION

The most important conclusion of this work is that free chromosome duplications of *C. elegans* can be lost somatically to produce genetic mosaics and that, at least for the four mutations studied, genetic mosaics can produce phenotypic mosaics. The patterns of mosaic expression were consistent with predictions from the known cell lineages, and no inconsistent patterns were found.

The view that *osm-1* behaves cell autonomously with respect to FITC staining of sensory neurons was drawn first from the finding of *unc-3 osm-1; Dp* hermaphrodites with only one of two phasmid neurons stained by FITC, a pattern that was attributable to somatic duplication loss. The FITC-staining patterns of Unc-3 and semi-Unc-3 animals arising from *unc-3 osm-1; Dp* zygotes also pointed to the cell autonomy of *osm-1*. This was most dramatically illustrated by the differences in staining between left and right amphids. Thus, among Unc-3 duplication mosaics, there were animals in which only left amphid neurons stained but no animals in which only right amphid neurons stained. Similarly, among semi-Unc-3 animals, there were several examples of nonstaining right amphids but no examples of nonstaining left amphids. These patterns were readily predicted from the known cell lineages of the amphid neurons (the lineages of these cells are not bilaterally symmetric).

The *daf-6* gene provides a good contrast to *osm-1* because it is clearly not cell autonomous with respect to FITC staining. The results fit nicely the evidence of ALBERT, BROWN and RIDDLE (1981) that the *daf-6* mutant has defective sheath cells, *i.e.*, it appears that absence of *daf-6+* from a sheath cell is sufficient to block staining of all sensory neurons of the corresponding sensillum. It is possible that a *daf-6* socket cell would also abolish staining. The results are not consistent with a requirement for both a socket and the sheath cell to lack *daf-6+* in order for the sensillum not to stain, however. The lineages of socket and sheath cells for the right phasmid, for example, diverge at AB.pr (Figure 1), but the socket and sheath cells of the right amphid also diverge at AB.pr. Hence, it would be impossible by a single event to generate duplication-free socket and sheath cells for one right sensillum without simultaneously affecting the other right sensillum; many examples of single nonstaining sensilla were found (Table 1). The cell lineages of the sheath (and socket) cells are bilaterally symmetric, which is in accord with the bilaterally symmetric staining patterns of the Unc-3 and semi-Unc-3 animals arising from *unc-3 daf-6; mnDp2* zygotes.

The results clearly indicate that the *unc-3* gene has a diffuse focus of action that is at least primarily localized among the descendants of AB.pl and AB.pr, which contribute about equally and additively to the Unc-3 phenotype. Thus, loss of *unc-3+* at AB.p confers an Unc-3 phenotype and loss at AB.pl or AB.pr confers a semi-Unc-3 phenotype. Losses among the descendants of AB.pl or AB.pr can also lead to a semi-Unc-3 phenotype (Table 3), but presumably these losses generally either occur earlier in the lineage than do the more frequent losses that affect sensillum staining in non-Unc animals (Table 1) or they involve double event losses. In any case, the high incidence of early losses among semi-Unc-3 animals suggests that more than one descendant of both AB.pl and AB.pr is responsible for the *unc-3* expression. Alternatively, *unc-3+* product may be made early and perdure to rescue critical cells lacking the *unc-3+* gene.

unc-3 animals move their heads normally, but they cannot propagate along their bodies normal dorsoventral bends necessary for smooth movement. They show this abnormality at hatching and retain it throughout development. Only one of 95 adult body wall muscle cells derive from AB.p; therefore, it is extremely unlikely that the focus of *unc-3* action is muscle cells. Figure 4 shows the cell

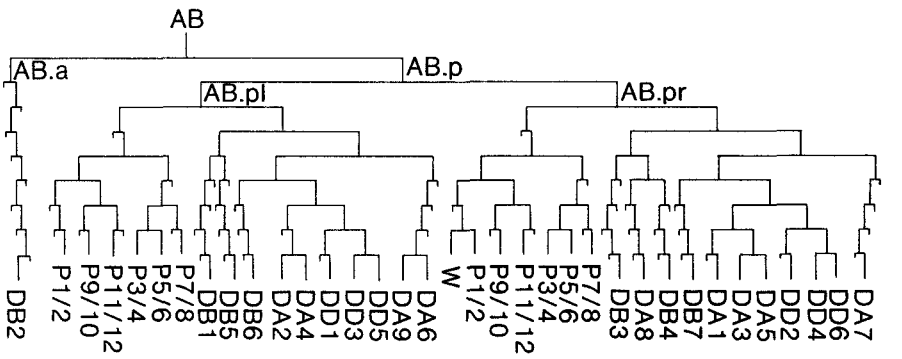

FIGURE 4.—Lineages of ventral and dorsal cord motor neurons (SULSTON *et al.* 1983). Lineage tree conventions are the same as for Figure 1. The names of all 22 motor neurons present in the newly hatched animal are given in the figure and begin with D. An additional 53 hermaphrodite cord motor neurons are generated through postembryonic lineages (not shown here; see SULSTON 1976; WHITE *et al.* 1976; SULSTON and HORVITZ 1977), which descend from 13 blast cells shown in the figure and called W and P1–P12. The cells P1–P12 are numbered (anterior to posterior) after their migration into the ventral cord, and, because there is some variability in the anterior-posterior order of a given left-right pair of P cells in the cord, each member of a pair is designated by the same symbol in the figure; thus, the two cells designated P1/P2 in the figure can be named P1 and P2 only after they have assumed their relative positions in the cord (SULSTON 1976; SULSTON and HORVITZ 1977).

lineages of all of the ventral and dorsal cord motor neurons, which drive the body muscles. All but one descend from AB.p; the lineages of these neurons make them, or a subset of them, prime candidates for the focus of *unc-3* action. (Various interneurons derive from both AB.a and AB.p; a subset of interneurons deriving from AB.p are thus also possible candidates for the focus of *unc-3* action.)

The predictions that were made in the *sup-10* experiments depended on the assumption that *sup-10* action is specific to muscle cells. This assumption follows from the conclusion of GREENWALD and HORVITZ (1980) that *unc-93(e1500)*, which *sup-10* suppresses, confers a muscle defect. The fact that the predictions were borne out by the results is taken as strong support for the correctness of the assumption. The strongest prediction was that wild-type-moving hermaphrodites arising from *unc-93; unc-3 sup-10; mnDp3* zygotes by virtue of somatic duplication loss would give only Unc-3 progeny. This result by itself only argues that the duplication was lost by a cell that is precursor to the germ line, *i.e.*, P_0-P_4 (see Figure 3). But P_0 is excluded because it is precursor to the AB lineage, which was shown to have retained the duplication, and P_4 can be excluded on the grounds that it produces only germ line cells. An argument against P_3 is that loss by D, which is not a precursor to the germ line, should be as effective. But, finally, the choice of P_1 depends on the assumption of muscle-specific action: loss of P_2 would leave 43 of 95 body muscle cells and all vulval and uterine muscles unsuppressed, which would be expected to give neither wild-type movement nor wild-type egg-laying ability; all of the wild-type duplication mosaics were good egg layers. I conclude that duplication loss in these animals did occur at P_1. It is noteworthy that absence of *unc-3*+ and *osm-1*+ from all

non-AB cells had no discernible phenotypic effect. Additional results with *sup-10* indicated that loss of the duplication somewhere between EMS and the precursor to the vulval and uterine muscles (Figure 3) enables the animal to lay eggs even though most of the body muscle cells carry *sup-10+* and are Unc-93. Depending on where the loss occurs, these animals may be mosaic for *sup-10+* in either body muscle cells or vulval and uterine muscle cells. Unfortunately, the abnormality in birefringence conferred by *unc-93* (GREENWALD and HORVITZ 1980) appears to be too subtle to use in identifying the phenotypes of individual cells.

An unexpected result in the *sup-10* experiments was the finding of variant duplications that had lost *sup-10+* but retained *unc-3+*. One possible means by which such altered duplications might arise would be through picking up the chromosomal *sup-10* mutation by recombination. But in at least two cases that mechanism seems unlikely, since the variant duplications in these cases were shown to be missing a nearby wild-type gene that was originally carried by the duplication as well as the chromosomes, *i.e.*, in these cases the loss most likely occurred through the formation of a deficiency in the duplication. Indeed, it is possible that all of the losses of *sup-10+* occurred through deficiency formation rather than recombination. These events are not unique to the *sup-10* region of *mnDp3*. I have observed simultaneous losses of more than one wild-type gene from *mnDp26* (HERMAN, MADL and KARI 1979), for example (unpublished observations), although the frequency of loss in that case was less than 10^{-4}. The frequency of formation of variant duplications apparently can be much higher than the usual mutation frequencies; thus, one should be aware of the possibility of such events when working with free duplications.

Duplication loss occurred at many somatic cell divisions in the experiments reported here. Approximate estimates of the frequency of loss per cell division can be made at different cell divisions for *mnDp2*. Loss at AB or AB.p occurred at a frequency of about 0.3% per division (Table 2), although loss at AB may be favored over loss at AB.p. If it is assumed that the action of *daf-6* is specific to sheath cells but not sockets, loss at AB.pl and AB.pr occurs at a frequency of about 0.2% per cell division (Table 3). Finally, if it is assumed that loss of the *mnDp2* could occur at any of eight cell divisions after AB.p (and prior to formation of a given sheath cell) to give rise to nonstaining sensillum in a non-Unc-3 animal (this would include semi-Unc-3 animals but they were not necessarily excluded from the data of Table 1), the overall average frequency of loss during these divisions would be about 0.4% per cell division. These are approximate estimates but suggest that the frequency of loss does not vary drastically over different parts of the lineage. *mnDp3* was lost much less frequently than *mnDp2*, perhaps because of its larger size (ALBERTSON and THOMSON 1982).

There are many other *C. elegans* genes for which mosaic analysis could provide useful information. As indicated in the introduction, free duplications covering several regions of the genome are already available, and it may be possible ultimately to cover virtually all regions. It will then be a matter of identifying suitable cell markers for particular free duplications in order to monitor duplication loss. A possible modification of the general scheme that has been discussed

involves using a duplication that carries a nonsense suppressor gene such as *sup-5* or *sup-7* (WATERSTON and BRENNER 1978; WATERSTON 1980; WILLS *et al.* 1983), which could then be used to generate animals mosaic for expression of any mutant gene suppressible by a single dose of the suppressor (R. WATERSTON, personal communication).

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LITERATURE CITED

ALBERT, P., S. BROWN and D. RIDDLE, 1981 Sensory control of dauer larva formation in *Caenorhabditis elegans*. J. Comp. Neurol. **198:** 435–451.

ALBERTSON, D. G. and J. N. THOMSON, 1976 The pharynx of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. (Biol. Sci.) **275:** 299–325.

ALBERTSON, D. G. and J. N. THOMSON, 1982 The kinetochores of *Caenorhabditis elegans*. Chromosoma **86:** 409–428.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

CHALFIE, M. and J. E. SULSTON, 1981 Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. Dev. Biol. **82:** 258–270.

CULOTTI, J. G. and R. L. RUSSELL, 1978 Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics **90:** 243–256.

DEPPE, U., E. SCHIERENBERG, T. COLE, C. KRIEG, D. SCHMITT, B. YODER and G. VON EHRENSTEIN, 1978 Cell lineages of the embryo of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **75:** 376–380.

GEHRING, W. J. (Editor), 1978 *Genetic Mosaics and Cell Differentiation*. Springer-Verlag, New York.

GREENWALD, I. S. and H. R. HORVITZ, 1980 *unc-93(e1500)*: a behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. Genetics **96:** 147–164.

HERMAN, R. K., 1978 Crossover suppressors and balanced recessive lethals in *Caenorhabditis elegans*. Genetics **88:** 49–65.

HERMAN, R. K., D. G. ALBERTSON and S. BRENNER, 1976 Chromosome rearrangements in *Caenorhabditis elegans*. Genetics **83:** 91–105.

HERMAN, R. K. and H. R. HORVITZ, 1980 Genetic analysis of *Caenorhabditis elegans*. pp. 227–261. In: *Nematodes as Biological Models*, Vol. 1, Edited by B. M. ZUCKERMAN. Academic Press, New York.

HERMAN, R. K., J. E. MADL and C. K. KARI, 1979 Duplications in *Caenorhabditis elegans*. Genetics **92:** 419–435.

HODGKIN, J., 1980 More sex determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

KIMBLE, J. and D. HIRSH, 1979 The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. **70:** 396–417.

LEWIS, J. A. and J. A. HODGKIN, 1977 Specific neuroanatomical changes in chemosensory mutants of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **172:** 489–510.

MENEELY, P. M. and R. K. HERMAN, 1981 Suppression and function of *X*-linked lethal and sterile mutations in *Caenorhabditis elegans*. Genetics **97:** 65–84.

MEYEROWITZ, E. M. and D. R. KANKEL, 1978 A genetic analysis of visual system development in *Drosophila melanogaster*. Dev. Biol. **62:** 112–142.

SIDDIQUI, S. S. and P. BABU, 1980 Genetic mosaics of *Caenorhabditis elegans*: a tissue-specific fluorescent mutant. Science **210:** 330–332.

SULSTON, J. E., 1976 Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. (Biol. Sci.) **275:** 287–297.

SULSTON, J. E., D. G. ALBERTSON and J. N. THOMSON, 1980 The *C. elegans* male: postembryonic development of nongonadal structures. Dev. Biol. **78:** 542–576.

SULSTON, J. E. and H. R. HORVITZ, 1977 Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

SULSTON, J. E., E. SCHIERENBERG, J. G. WHITE and J. N. THOMSON, 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol., **100:** 64–119.

TRENT, C., N. TSUNG and H. R. HOROVITZ, 1983 Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics **104:** 619–647.

WARD, S., 1976 The use of mutants to analyze the sensory nervous system of *Caenorhabditis elegans*. pp. 365–382. In: *The Organization of Nematodes*, Edited by N. A. CROLL. Academic Press, New York.

WARD, S., N. T. THOMSON, J. G. WHITE and S. BRENNER, 1975 Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **160:** 313–338.

WARE, R. W., D. CLARK, K. CROSSLAND and R. L. RUSSELL, 1975 The nerve ring of the nematode *Caenorhabditis elegans*: sensory input and motor output. J. Comp. Neurol. **162:** 71–110.

WATERSTON, R. H., 1980 A second informational suppressor, *sup-7 X*, in *Caenorhabditis elegans*. Genetics **97:** 307–325.

WATERSTON, R. H. and S. BRENNER, 1978 A suppressor mutation in the nematode acting on specific alleles of many genes. Nature **275:** 715–719.

WHITE, J. G., E. SOUTHGATE, J. N. THOMSON and S. BRENNER, 1976 The structure of the ventral nerve cord of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. (Biol. Sci.) **275:** 327–348.

WILLS, N., R. F. GESTELAND, J. KARN, S. BOLTEN AND R. H. WATERSTON, 1983 Transfer RNA-mediated suppression of nonsense mutations in *C. elegans*. Cell **33:** 575–583.

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